

AMENDMENT

Amendments to the Specification:

Please delete the previously filed “Sequence Listing” and insert the uploaded substitute “Sequence Listing” filed herewith.

Please replace the paragraph at page 35, line 25 bridging to page 36, line 2 with the following amended paragraph:

Five samples for each tissue type were collected for each of three age groups; over 65 years. 50 to 65 years and under 50 years. Genomic DNA was extracted from the tissue samples using Qiagen Genomic-Tip 500/G columns. The five DNA samples from each tissue type and each age group were pooled and experiments were performed as follows. DNA was digested with RsaI to generate smaller DNA fragments before digestion with MspI and HpaII, two restriction enzymes with different sensitivities to cytosine methylation. Then MS AP-PCR was performed as previously described by Liang et al. The digested DNA was amplified using these sets of primers: G1, 5'-GCGCCGACGT-3' (SEQ ID NO:42); G5, 5'-TGCGACGCCG-3' (SEQ ID NO:43); APBS5, 5'-CTCCCCACGCG-3' (SEQ ID NO:44). After amplification fragments were separated on polyacrylamide gels and those exhibiting a pattern of differential methylation were eluted from the gel, cloned into vectors and sequenced as outlined above. Identification of sequences was performed by BLAST searches in Genbank.

Please replace the paragraph at page 36, line 30 bridging to page 37, line 11 with the following amended paragraph:

Bisulfite genomic sequencing was performed for the ALX4 gene in order to confirm the results obtained by MethylLight™ analysis. Briefly, bisulfite treated genomic DNA from 4 colon cancers and matched normal colon mucosa was amplified with primers specific for a fragment of the ALX4 gene containing 39 CpG sites and spanning the region that was analysed with the Methylight™ assay: ALX4_bis1, 5'-TGAATAGGGTGATATTTAGTTAGG-3' (SEQ ID NO:76); ALX4_bis2, 5'-ATAAATCATCCAAAACCTCTA-3' (SEQ ID NO: 60). PCR was carried out in a reaction mixture (25 µl) containing 7 µl of DNA, 0.2 mM dNTPs, 1 µM primers, and 0.25 units of DyNAzyme EXT DNA Polymerase (FimlZyines). Amplification was performed using the following condition: 94°C for 2 min, followed by 36 cycles (94°C for 1 min, 45°C for 1 min, 72°C for 2 min) and then 72°C for 10 minutes. PCR products were separated on 1 % agarose gel, stained with ethidium bromide and visualized with an UV transilluminator. DNA fragments of interest were cloned into a plasmid vector with the TOPO TA cloning kit (Invitrogen, Carlsbad, CA) according to manufacturer's recommendations and sequence was confirmed by automated sequencing.

Please replace Table 1 at page 44 with the following amended Table 1:

Table 1. List of primers and probes used for Methylight analysis

| Gene | forward primer (5' - 3') | reverse primer (5' - 3') | probe sequence (5' - 3') |
|-----------------|--|--|--|
| ALX4 | CGCGGTTTCGATTTAACGAT <u>(SEQ ID NO:21)</u> | ACTCCGACTTAACCCGACGAT <u>(SEQ ID NO:22)</u> | 6FAM- CGACGAAATTCTTAACGCAACCGCTT AA-BHQI <u>(SEQ ID NO:23)</u> |
| Caveol in 2 | TTTCGGATGGGAACGGTGTA <u>(SEQ ID NO:24)</u> | CTCCCACCGCCGTTACC <u>(SEQ ID NO:25)</u> | 6FAM- CCCGTCCTAACCGTCCGCCCT-BHQI <u>(SEQ ID NO:26)</u> |
| DAPK | TCGTCGTCGTTCGGTTAGTT <u>(SEQ ID NO:27)</u> | CCCTCCGAAACGCTATCGA <u>(SEQ ID NO:28)</u> | 6FAM- CGACCATAAACGCCAACGCCG-BHQI <u>(SEQ ID NO:29)</u> |
| TPEF | TTTTTTTCGGACGTCGTTG <u>(SEQ ID NO:30)</u> | CCTCTACATACGCCGCGAAT <u>(SEQ ID NO:31)</u> | 6FAM- AATTACCGAAAACATCGACCGA- BHQI <u>(SEQ ID NO:32)</u> |
| p16 / IN K4A | TGGAATTTCGGTTGATTGGTT <u>(SEQ ID NO:33)</u> | AACAACGTCCGCACCTCCT <u>(SEQ ID NO:34)</u> | 6FAM-ACCCGACCCCGAACCGCG- BHQI <u>(SEQ ID NO:35)</u> |
| APC | GAACCAAAACGCTCCCCAT <u>(SEQ ID NO:36)</u> | TTATATGTCGGTTACGTGCGTTAT AT <u>(SEQ ID NO:37)</u> | 6FAM- CCCGTCGAAAACCCGCCGATT- BHQI <u>(SEQ ID NO:38)</u> |
| TIMP3 | GCGTCGGAGGTTAAGGTTGT <u>(SEQ ID NO:39)</u> | CTCTCCAAAATTACCGTACGCG <u>(SEQ ID NO:40)</u> | 6FAM-AACTCGCTCGCCGCCGAA- BHQI <u>(SEQ ID NO:41)</u> |
| Caveol in | TTTCGGATGGGAACGGTGTA <u>(SEQ ID NO:24)</u> | CTCCCACCGCCGTTACC <u>(SEQ ID NO:25)</u> | 6FAM- CCCGTCCTAACCGTCCGCCCT-BHQI <u>(SEQ ID NO:26)</u> |